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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

INVENTOR(S)/APPLICANT(S)

Given Name (last name, first name, and middle initial [if any])			Residence (City and State or Foreign Country)
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No Additional inventors are being named

TITLE OF THE INVENTION

(280 characters max)

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ENCLOSED APPLICATION PARTS

☒ Specification — Number of Pages: 5

☐ Drawings (☐ Formal; ☐ Informal) — Number of Sheets: (Figs.)

☐ Other documents (specify):

This invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are.

METHOD OF PAYMENT

☒ A check in the amount of $80.00 (Small Entity fee) is enclosed to cover the filing fees of the Provisional application.

☐ The Commissioner is hereby authorized to charge $75.00 to cover the filing fees of the Provisional application to Deposit Account No., Reference No.

☒ The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No. 50-0311, Reference No. 18475-044.

Respectfully submitted,

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT
SEND TO: *BOX PROVISIONAL APPLICATION, Assistant Commissioner for Patents, Washington, DC 20231*

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IDENTIFICATION OF FETAL CELLS IN MATERNAL BLOOD USING MATERNAL ANTIBODIES TO FETAL ANTIGENS

BACKGROUND OF THE INVENTION

The invention relates to prenatal testing.

Fetal testing for chromosomal abnormalities is often performed on cells obtained using amniocentesis or Chorionic-villus sampling (CVS). Amniocentesis is a procedure used to retrieve fetal cells from the fluid that surrounds the fetus. This relatively invasive procedure is performed after the 12th week of pregnancy holds about a half percent increased risk of miscarriage. CVS is relatively less invasive and can be done as early as 10 and a half weeks of pregnancy. The latter procedure increases the risk of a miscarriage by about 1 percent.

SUMMARY OF THE INVENTION

Fetal cells are identifed and retrieved from maternal blood using maternal antibodies, which are produced by the mother against paternally-inherited fetal antigens present on the surface of a fetal cell. The paternally-inherited antigens are unique fetal cell markers, which are targeted for the isolation of fetal cells from maternal blood.

The method of identifying and/or isolating a fetal cell from maternal peripheral blood is carried out by detecting maternal antibodies that are specifically bound to the fetal cells among the nucleated cells isolated from maternal blood. A fetal cell-antibody complex is detected. The complex contains an a maternally-produced antibody specific for a paternally-inherited fetal antigen bound to the surface of a fetal cell. The maternal antibodies have come into contact with fetal cells during their time spent in the maternal plasma, and are therefore bound to fetal cells in the cell preparations from maternal blood. To further assure the binding of maternal antibodies to all fetal cells in the preparation, the cell preparation can be further incubated under suitable conditions with maternal plasma, or the maternal antibodies can be isolated from the plasma and contacted with the cell preparation under suitable conditions. The method is used to enrich (increase the proportion of) fetal cells in cell preparations from maternal blood.

Maternal antibodies may also bind to a small number of maternal cells in the circulation. Additional isolation steps are optionally carried out to achieve fetal cell purification.

Antibodies may be bound to fetal cells already at the time of blood collection, due to the exposure of fetal cells to maternal plasma from the time the cells crossed over into the maternal circulation. Alternatively, a preparation of peripheral blood mononuclear cells is contacted with maternal plasma, an antibody-containing fraction of said plasma, or a purified antibody preparation isolated from said plasma, under conditions permitting formation of an antibody complex with the fetal cells. In this case, cell preparations isolated from maternal blood are optionally treated to unmask surface bound maternal antibodies, or to increase HLA expression. The maternal antibody-bound fetal cell complex is identified with a second antibody or other molecule that binds to human antibodies. The isolation of fetal cells thus detected is achieved by a fluorescence label on the secondary antibody, followed by fluorescence-activated cell sorting. Alternatively, the secondary label can be coupled to magnetizable beads and the fetal cells isolated with a magnet.

In the case that the fetus is male, the isolation of fetal cells can be confirmed using a Y chromosome marker. Alternative methods are available in case the fetus is female.

DETAILED DESCRIPTION

Fetal cells isolated from maternal blood represent a non-invasive source of fetal DNA for prenatal genetic screening and diagnosis of fetal abnormalities or a predisposition thereto. This approach is hampered by the extreme scarcity of such fetal cells in the maternal circulation. Another problem associated with existing methods is the lack of a marker that identifies all fetal cells rather than a sub-population. A pan-fetal antibody has not been available. This invention solves the problem of earlier methods by utilizing a reagent that targets all fetal cells.

In the course of a normal pregnancy, the mother mounts a humoral immune response against paternally-inherited fetal antigens. This means that maternal antibodies are present in maternal plasma and bind specifically to every fetal cell expressing a paternally-inherited HLA antigen. The antibodies are unique to a particular pregnancy except that they may be useful to identify fetal cells in subsequent pregnancies in which the fetus has the same father.

Secondary labels and cell isolation

Maternal antibodies bound to cells can be detected using anti-human antibodies or their Fab fragments, as well as other molecules such as protein A. These secondary labels may be conjugated with fluorescent molecules, for cell isolation via fluorescence activated cell sorting,

Alternatively, the secondary label can be coupled to magnetizable beads and the cells isolated by a magnet.

Cell treatment for antibody labeling

Maternal antibodies on fetal cells may be masked and unavailable for binding of secondary label, due to unknown mechanisms that protect fetal cells from maternal immune attack. To address potential masking, nucleated cells from maternal blood are isolated and then treated to unmask bound antibodies to allow labeling with a detectable marker as described above.

Purpose of secondary exposure of cell preparations to plasma or purified antibodies

It is possible that maternal antibodies bound to a fetal cell fix complement and destroy some of the fetal cells to which they bind while in the maternal circulation. Thus, only fetal cells that have not bound many maternal antibodies are available for isolation. These cells can be exposed to maternal plasma or antibody preparations therefrom under conditions that does not allow complement lysis, e.g., in the presence of a chelating agent such as EDTA.

Maternal antibody fraction

Plasma or serum obtained from a pregnant woman is used as a source of maternal antibodies. To avoid complement-mediated cell lysis and to allow cell incubations at high antibody concentrations, antibodies from the plasma are enriched or purified by standard methods, e.g., ammonium sulfate precipitation or affinity chromatography.

Cell amplification and subsequent labeling of fetal cells

Instead of labeling cells from maternal blood immediately after extraction, the cells are optionally cultured and amplified. Following culture, fetal cells are identified using the incubation with maternal plasma antibodies as described above.

Example of enrichment of fetal cells from maternal blood

Blood samples (10 ml) from three pregnant woman was obtained and processed as follows:

Nucleated cells were isolated using a density gradient. The isolated cells were exposed for 30 min to a mixture of goat F(ab')2 anti-human IgG + goat (Fab')2 anti-human IgM, both of which were conjugated with the fluorescent dye phycoerythrin (PE). The cells were washed and suspended in PBS with 1% formaldehyde, DNA-specific dye Hoechst 33342 and 0.05% Triton X100.

The cell sample was subjected to fluorescence-activated cell sorting (FACS) to flow-sort fluorescent cells (Hoechst +, PE+). The PE+ cells, i.e., cells that bound the anti-antibody-antibody, represented a small proportion of all cells (< 1:100,000).

The flow sorted cells (on microscope slides) were labeled by fluorescence *in situ* hybridization (FISH), using probes specific for Y and X -chromosome. The numbers of cells containing a Y signal (= male, fetal) was quantified. One maternal blood cell preparation contained 10 fetal cells, the other 5.

Following FACS, the concentration of fetal cells in the mononuclear cell population was at least 1 in 1000.

What is claimed is:

1. A method of identifying a fetal cell in a sample of maternal blood, comprising detecting a cell complex, said complex comprising a mononuclear cell bound to an antibody, wherein detection of said complex indicates the presence of a fetal cell.
2. The method of claim 1, wherein said antibody is a maternally-produced antibody specific for a paternally-inherited fetal antigen.
3. The method of claim 1, wherein said complex is detected by contacting a population of maternal peripheral blood mononuclear cells with a ligand that specifically binds to a human antibody.
4. The method of claim 3, wherein said ligand comprises a detectable label.
5. The method of claim 4, wherein said detectable label is a fluorescent molecule.
6. The method of claim 1, wherein said complex is detected by by fluorescence activated cell sorting.
7. The method of claim 4, wherein said detectable label further comprises a magnetizable particle.
8. The method of claim 7, wherein said method further comprises contacting said magnetizable particle with a magnet.
9. A method of identifying a fetal cell in a sample of maternal blood, comprising contacting isolated maternal peripheral blood mononuclear cells with an isolated maternally-produced antibody, wherein said antibody binds to a paternally-inherited antigen on said fetal cell.

10. A sample of maternal peripheral blood mononuclear cells (MPBMC), comprising an enriched population of fetal cells, wherein the concentration of fetal cells in said sample is at least 1 in 1000 MPBMC.